

Amendments to the Specification

Please replace the first paragraph of the specification with the following paragraph.

This application is a § 371 application of PCT/EP2003/011810, filed 23 October 2003, which claims the benefit of Great Britain Application Number GB 0224688.2, filed 23 October 2002.

Please replace page 1 with amended page 1 appended hereto.

Please replace the first paragraph on page 4 with the following amended paragraph.

Most preferably a plastic[[s]] microtitre plate is coated with a suitable immunoglobulin according to well known methods in the art.

Please replace the last paragraph on page 4 with the following amended paragraph.

The mixing of the antigen sample with the antibody is carried out in the presence of a basic buffer, which is any suitable buffer having a pH greater than 7. Preferably the buffer has a pH of greater than 8, more preferably having a pH of greater than 8.5 and most preferably having a pH of substantially 9. Preferably the pH is between 7 and 12, more preferably between 8 and 11, most preferably between 8 and 10. The pH can be adjusted to take account of the specific antigen being tested, to optimise the method – that is, to optimise binding and/or minimise the effect of aluminium hydroxide on the assay. Preferably the buffer contains 1% Tween[™] detergent, or functional equivalent thereof. Most preferably the buffer is DEA 0.2M, HCl 0.2M at pH9 with 1% Tween[™] added, preferably for use in the detection of hepatitis B surface antigen.

Please replace the paragraphs identified with the numerals “3” and “4” on page 7 with the following paragraphs.

- 3 After a washing step (in 150 mM NaCl, 0.05% Tween™) the solid phase was blocked with PBS 1% BSA buffer for 1 hour at 37°C, then washed with NaCl 150 mM + Tween 20™~~tween-20~~ 0.05% solution.
- 4 The detection of anti-HBs/HBsAg complex was then performed by addition of a pool of 3 anti-HBs mouse monoclonal antibodies diluted at 1µg/ml in PBS, 0.2% BSA, 0.1% Tween™ and 4% newborn calf serum and then incubated for 1 hour at 37°C. Excess antibodies were removed by washing and then plates were incubated for 30 min at room temperature (RT) with agitation with a biotin-conjugated anti-mouse Ig (from Prosan™). After washing, the Amdex™ streptavidin horseradish peroxidase complex (from Amersham™) was added to the wells (30min at RT with agitation) . Plates were then washed and incubated for 20 min with agitation with a solution of o-phenylenediamine (Sigma™) 0.04%, H₂O₂ 0.03%, 0.1% Tween 20™~~tween-20~~, 0.05M citrate buffer pH4.5. The reaction was stopped with H₂SO₄ 2N and read at 490 and 630 nm. The signal obtained at 630nm is subtracted from that at 490nm and can be used to calculate HBsAg concentrations in the sample through reference to a standard by SoftmaxPro (concentration expressed in µg/ml).

Please replace the paragraph indicated by the fourth bullet point on page 9 with the following paragraph.

- The detection of anti-HBs/HBsAg complex was then performed by addition of a pool of 3 anti-HBs mouse monoclonal antibodies diluted at 1µg/ml in PBS, 1% BSA, 0.1% Tween 20™ and 4% newborn calf serum and then incubated for 1 hour at 37°C. Excess antibodies were removed by washing and then plates were incubated for 30 min at room temperature (RT) with agitation with a biotin-conjugated anti-mouse Ig (from Prosan™) diluted in PBS 1% BSA 0.1% Tween

20TM. After washing, the AmdexTM streptavidin horseradish peroxidase complex (from AmershamTM) was added to the wells diluted in PBS 1% BSA 0.1% Tween 20TM (30min at RT with agitation) . Plates were then washed and incubated for 20 min with agitation with a solution of o-phenylenediamine (SigmaTM) 0.04%, H₂O₂ 0.03%, 0.1% ~~Tween 20TM~~ ~~tween 20~~, 0.05M citrate buffer pH4.5. The reaction was stopped with H₂SO₄ 2N and read at 490 and 630 nm. HBsAg concentrations in samples were calculated from a reference by SoftmaxPro and expressed in µg/ml.

Please replace the abstract with the following amended abstract.

The invention relates to a method for the detection of an antigen, ~~the antigen being~~ in the presence of aluminium hydroxide, and kits ~~comprising instructions and components-suitable~~ for carrying out the disclosed ~~said~~-method.